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EFFECT OF ALKYL-N-PHENYLCARBAMATES ON PHOTOCHEMICAL ACTIVITY OF SPINACH CHLOROPLASTS

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Several derivatives of N-phenylcarbamate are found wide application in agriculture as herbicides [1]. This study is aimed to investigate the effect of alkyl-N-phenylcarbamates (APC) on photosynthetic electron transport (PET) in spinach chloroplasts, to determine their site of action in the photosynthetic apparatus of spinach chloroplasts and to find correlations between their structure and biological activity.

Thirteen alkyl-N-phenylcarbamates (alkyl = methyl - octyl) were prepared by reductive carboxylation of nitrobenzene with carbon oxide and alkanethiol [2]. The inhibition of PET in spinach chloroplasts in the presence of APC has been monitored spectrophotometrically i/ by the rate of photoreduction of 2,6-dichlorophenol-indophenol (DCPIP) (for monitoring of PET through photosystem (PS) 2), ii/ by the rate of photooxidation of HDCPIP (for monitoring of PET through PS 1). For determination of the site of APC action in the photosynthetic apparatus of spinach chloroplasts EPR spectroscopy has been used.

The PET through PS 2 was inhibited by the studied compounds and the corresponding IC_{50} values (molar concentrations of inhibitors causing 50 % decrease of biological activity with respect to the untreated control sample) varied in the range from 10 (R = CH_3) to 208 μmol dm^{-3} (R = $CH_2CH(CH_3)_2$). With respect to the fact that the site of DCPIP action in the photosynthetic chain of electron transport is the plastoquinone pool (PQ), the decreased rate of DCPIP photoreduction in the presence of APC suggests that APC cause damage of PS 2. On the other hand, it has been found that APC did not decrease the rate of HDCPIP photoxidation. Consequently it can be concluded that PS 1 is not damaged by APC.

For more precise determination of the site of action of APC in the chain of PET an artificial electron donor 1,5-diphenylcarbazide (DPC) acting in Z/D intermediate which is located on the donor side of PS 2, has been used (3). This intermediate secures the electron transport from the oxygen evolving complex to the core of PS 2 (P680). With respect to the fact that DPC produces only partial restoration of PET in chloroplasts inhibited by APC (up to 50 %) we can assume that the inhibitory site of APC action is a part of the electron transport chain between the site of action of DPC and DCPIP, i.e. the part from the intermediate Z/D through P680, pheophytin, the first and the second quinone acceptor Q_A and Q_B up to plastoquinone pool PQ.

EPR spectroscopy is a method enabling to determine the interactions of photosynthesis-inhibiting compounds with photosynthetic centres. Intact chloroplasts of higher plants exhibit at room temperature EPR signals in the region of free radicals (g 2.0), known as signal I and signal II which are connected with both photosystems PS 1 and PS 2 (4). In the presence of APC the intensity of the slow constituent of signal II

(signal II_{slow}) shows a decrease. With respect to the fact that signal II_{slow} belongs to the intermediate D, i.e. to the tyrosine radical (Tyr_D) which is situated in the position 161 in D_2 protein on the donor side of PS 2 (5), it can be assumed that APC interact with the intermediate D. Due to this interaction restriction of the electron transport through the photosynthetic transport chain occurs causing restricted reduction of P700 what is in the light in oxidized form. This was reflected in EPR spectra of APC-treated chloroplasts by a great intensity increase of signal I. The fast constituent of signal II (signal $II_{very\ fast}$) belonging to the intermediate Z, i.e. to the tyrosine radical (Tyr_Z) which is situated in the position 161 in D_1 protein on the donor side of PS 2 (5), was not affected by APC. The interaction of APC with the intermediate D was indirectly confirmed also by the above described experiment with DPC in which the photoreduction of DCPIP was not completely restored by addition of DPC to chloroplasts inhibited by APC.

The most active inhibitor was methyl derivative (IC_{50} = 10 μmol dm^{-3}) and with increasing lipophilicity of the studied compounds the photosynthesis-inhibiting activity showed a linear decrease for compounds with linear alkyl substituent. The inhibitory activity of compounds with branched alkyl substituent (R = isopropyl, tert. butyl, isobutyl) was lower than that of their linear isomers. Their lower effectiveness can be connected with the fact that for the achievement of the site of action in the photosynthetic apparatus the branched substituent represent higher steric hindrance than its linear isomers. Our findings differ from the results of Hansch and Deutsch which found that the inhibition of Hill reaction in chloroplasts produced by ethyl and isopropyl APC derivatives with different substituents on the benzene ring (in positions 3 and 4) shows an increase with increasing lipophilicity of the compounds [6]. The predominant effect of APC on the intermediate D can be explained by their relatively high lipophilicity. Intermediate D is namely located in less polar regions of thylakoid membranes than the intermediate Z (5). On the other hand, previously studied less hydrophobic derivatives of phenylcarbamic acid used in the form of ammonium salts interacted not only with the intermediate D, but also with the intermediate Z and with the manganese cluster (7) situated in the oxygen evolving complex which are located in the polar regions of thylakoid membranes.

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